FORM PTO-1390 (REV 10-94) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	9997.34USWO
	U S APPLICATION NO (If known, see 37 C F R 1 5) Unknown 09/936011

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/02176	7 March 2000	12 March 1999

TITLE OF INVENTION

METHOD FOR INDUCING VIRAL RESISTANCE INTO A PLANT

APPLICANT(S) FOR DO/EO/US

JONARD et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An unsigned oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98, Form 1449, 7 references, International Search Report.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND of SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: International Preliminary Examination Report

U.S. APPLICATION NO (If known, see 37 C F R 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Unknown 09/936011	PCT/EP00/02176	9997.34USWO

17. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):		
Search Report has been prepared by the EPO or JPO **$860.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(1)) **$690.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$710.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO **$1000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	22 -20 =	2	X **$18.00**	**$ 36.00**	
Independent claims	2 -3 =		X **$80.00**	**$**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	**$**	
TOTAL OF ABOVE CALCULATIONS =				**$896.00**	
Reduction by 1/2 for filing by small entity, if applicable. Small entity status is claimed pursuant to 37 CFR 1.27				**$**	
SUBTOTAL =				**$896.00**	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f). +				**$**	
TOTAL NATIONAL FEE =				**$896.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				**$**	
TOTAL FEES ENCLOSED =				**$896.00**	
				Amount to be: refunded	**$**
				charged	**$**

a. [X] Check(s) in the amount of $896.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
John J. Gresens
MERCHANT & GOULD
P.O. Box 2903
Minneapolis, MN 55402-0903

SIGNATURE: 

NAME: John J. Gresens

REGISTRATION NUMBER: 33,112

S/N unknown PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	JONARD et al.	Docket No.:	9997.34USWO
Serial No.:	unknown	Filed:	concurrent herewith
Int'l Appln No.:	PCT/EP00/02176	Int'l Filing Date:	7 March 2000
Title:	METHOD FOR INDUCING VIRAL RESISTANCE INTO A PLANT		

CERTIFICATE UNDER 37 CFR 1.10
'Express Mail' mailing label number: EL669942610US
Date of Deposit: 5 September 2001
I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C . 20231.

By: Omesh Singh
Name: Omesh Singh

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D. C. 20231

Dear Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment, that is based on claims amended in prosecution of the international application and published in the International Preliminary Examination Report, a copy of which is enclosed herewith:

IN THE ABSTRACT

Insert the attached Abstract page into the application as the last page thereof.

IN THE SPECIFICATION

A courtesy copy of the originally-filed PCT specification is enclosed herewith, but the World Intellectual Property Office (WIPO) copy should be relied upon if it is already in the U.S. Patent Office.

IN THE CLAIMS

Please amend the following claims as indicated below. A marked-up copy of all claims is attached for reference.

3. (amended) Method according to claim 1, characterized in that the group I virus is selected from the group consisting of hordeiviruses, benyviruses, pecluviruses and pomoviruses, preferably selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus.

4. (amended) Method according to claim 1, characterized in that the plant cell is a stomatal cell.

5. (amended) Method according to claim 1, characterized in that the plant is selected from the group consisting of sugar beet, potato, barley or peanut.

6. (amended) Method according to claim 1, characterized in that the virus is BNYVV, the nucleotide sequence of TGB2 of said virus is comprised between the nucleotide 3287 and 3643 of the 5' strand of genomic or subgenomic RNA 2 of the BNYVV and the plant is a beet, preferably a sugar beet (*Beta vulgaris*).

7. (amended) Method according to claim 1, characterized in that the regulatory sequence comprises a promoter sequence or a terminator sequence active in a plant.

10. (amended) Method according to claim 7, characterized in that the promoter sequence is a promoter which is capable of being active mainly into the root tissues of plants, such as the par promoter of the haemoglobin gene from Perosponia andersonii.

13. (amended) Transgenic plant according to claim 11, characterized in that the virus is selected from the group consisting of hordeiviruses, benyviruses, pecluviruses and pomoviruses, preferably selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus.

14. (amended) Transgenic plant according to claim 11 being a plant selected from the group consisting of sugar beet, potato, barley or peanut.

15. (amended) Transgenic plant according to claim 11, characterized in that the transgenic plant being a beet, preferably a sugar beet (*Beta vulgaris*) the virus is BNYVV and the

nucleotide sequence of TGB2 of said virus is comprised between the nucleotides 3287 and 3643 of the 5' strand of genomic or subgenomic RNA 2 of BNYVV or its corresponding cDNA.

16. (amended) Transgenic plant according to claim 11, characterized in that the regulatory sequence comprises a promoter sequence and a terminator sequence active in a plant.

17. (amended) Transgenic plant according to claim 11, characterized in that the regulatory sequence(s) comprise a promoter sequence which is a constitutive or a foreign promoter sequence.

19. (amended) Transgenic plant according to claim 17, characterized in that the promoter sequence is a promoter which is capable of being active mainly into root tissues, such as the par promoter of the haemoglobin gene from *Perosponia andersonii*.

20. (amended) Transgenic plant according to claim 11, characterized in that it further carries natural tolerance to Group I viruses.

21. (amended) Transgenic plant according to claim 11, characterized in that it further comprises a pesticide, herbicide or fungicide resistance, preferably a resistance selected from the group consisting of nematode resistance, glyphosate resistance, glufosomate resistance and/or acetochloride resistance.

22. (amended) Transgenic plant tissue selected from the group consisting of fruit, stem, root, tuber, seed of a plant according to claim 11.

REMARKS

The above preliminary amendment is made to remove multiple dependencies from claims 3-7, 10, 13-17, 19-22, .

A new abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

Applicant respectfully requests that the preliminary amendment described herein be entered into the record prior to calculation of the filing fee and prior to examination and consideration of the above-identified application.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicant's primary attorney-of record, John J. Gresens (Reg. No. 33,112), at (612) 371.5265.

Respectfully submitted,

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Dated: 5 September 2001

By _____
John J. Gresens
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JJG/kjr

ABSTRACT OF THE DISCLOSURE

The present invention concerns a method for inducing resistance to a virus comprising a TGB2 sequence into a cell plant or a plant, comprising the following steps: preparing a nucleotide construct comprising a nucleotide sequence corresponding to at least 70% of the nucleotide sequence to TGB2 of said virus or its complementary cDNA, being operably linked to one or more regulatory sequence(s) active in a plant, transforming a plant cell with the nucleotide construct, and possibly regenerating a transgenic plant from the transformed plant cell. The present invention is also related to the plant obtained.

MARKED UP COPY OF CLAIMS

3. Method according to [the]claim 1[or 2], characterized in that the group I virus is selected from the group consisting of hordeiviruses, benyviruses, pecluviruses and pomoviruses, preferably selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus.

4. Method according to [any of the preceding]claim[s] 1, characterized in that the plant cell is a stomatal cell.

5. Method according to [any of the preceding]claim[s] 1, characterized in that the plant is selected from the group consisting of sugar beet, potato, barley or peanut.

6. Method according to claim 1[or 2], characterized in that the virus is BNYVV, the nucleotide sequence of TGB2 of said virus is comprised between the nucleotide 3287 and 3643 of the 5' strand of genomic or subgenomic RNA 2 of the BNYVV and the plant is a beet, preferably a sugar beet (*Beta vulgaris*).

7. Method according to [any of the preceding]claim[s] 1, characterized in that the regulatory sequence comprises a promoter sequence or a terminator sequence active in a plant.

10. Method according to [any of the]claim 7[to 9], characterized in that the promoter sequence is a promoter which is capable of being active mainly into the root tissues of plants, such as the par promoter of the haemoglobin gene from Perosponia andersonii.

13. Transgenic plant according to [the]claim 11[or 12], characterized in that the virus is selected from the group consisting of hordeiviruses, benyviruses, pecluviruses and pomoviruses, preferably selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus.

14. Transgenic plant according to [the]claim[s] 11[to 13] being a plant selected from the group consisting of sugar beet, potato, barley or peanut.

15. Transgenic plant according to [the]claim[s] 11[or 12], characterized in that the transgenic plant being a beet, preferably a sugar beet (*Beta vulgaris*) the virus is BNYVV and the

nucleotide sequence of TGB2 of said virus is comprised between the nucleotides 3287 and 3643 of the 5' strand of genomic or subgenomic RNA 2 of BNYVV or its corresponding cDNA.

16. Transgenic plant according to [any of the preceding]claim[s] 11[to 15], characterized in that the regulatory sequence comprises a promoter sequence and a terminator sequence active in a plant.

17. Transgenic plant according to [any of the preceding]claim[s] 11[to 16], characterized in that the regulatory sequence(s) comprise a promoter sequence which is a constitutive or a foreign promoter sequence.

19. Transgenic plant according to claim 17[or 18], characterized in that the promoter sequence is a promoter which is capable of being active mainly into root tissues, such as the par promoter of the haemoglobin gene from *Perosponia andersonii*.

20. Transgenic plant according to [any one of the]claim[s] 11[to 19], characterized in that it further carries natural tolerance to Group I viruses.

21. Transgenic plant according to [any one of the]claim[s] 11[to 20], characterized in that it further comprises a pesticide, herbicide or fungicide resistance, preferably a resistance selected from the group consisting of nematode resistance, glyphosate resistance, glufosomate resistance and/or acetochloride resistance.

22. Transgenic plant tissue selected from the group consisting of fruit, stem, root, tuber, seed of a plant according to [any of the]claim[s] 11[to 21].

METHOD FOR INDUCING VIRAL RESISTANCE INTO A PLANT

Field of the invention

The present invention is related to a method for inducing viral resistance into a cell and a plant, especially BNYVV-resistance into a sugar beet cell and plant.

Background of the invention and state of the art

The widespread viral disease of the sugar beet plant (*Beta vulgaris*) called Rhizomania is caused by a benyvirus, the beet necrotic yellow vein virus (BNYVV) (23, 24) which is transmitted to the root of the beet by the soilborne fungus *Polymyxa betae* (25).

The disease significantly affects acreages where the sugar beet plant is grown for industrial use in Europe, USA and Japan and is still in extension in several places in Western Europe (26, 27). As there exists no practical method to effectively control the spread of the virus at a large scale by chemical or physical means (28), neither in the plants nor in the soil, the main focus has been to identify natural sources of resistance within the sugar beet germplasm and to develop by breeding, varieties of sugar beet plants expressing the resistance genes. A variety of such tolerance genes to the virus have been identified and, some have been successfully used in the breeding of commercial sugar beet varieties (29, 30, 31).

Only the use of BNYVV-resistant or tolerant varieties will enable farmers to grow sugar beet plants in BNYVV-infected areas where the sugar beet plant is an essential component of the crop rotation and contributes significantly to the grower's income.

A number of detailed studies have shown that a difference in susceptibility to the BNYVV-infection among sugar beet genotypes or varieties, generally reflect difference in the diffusion or translocation of the virus in the root tissues (32).

However, there are still few reports which indicate clearly that the tolerance genes, even from differing sources of sugar beet germplasm or wild relatives germplasm (33), would provide distinct mechanisms of resistance. Such a situation would represent a more manageable situation to design long lasting BNYVV-resistance strategies.

Since 1986, a number of reports and publications have described the use of isolated viral gene sequences expressed in plants to confer a high level of tolerance against the virus or even to confer a broad spectrum type of resistance against a number of related viruses (34, 35, 36). One of the most documented viral resistance strategy based on genetic engineering, in many cultivated species such as potato, squash, cucumber or tomato, is the use of the viral gene sequence which under the control of plant regulatory elements, encodes the coat-protein of the target virus (37).

However, for coat-protein mediated resistance, the expression of a certain level of resistance in the transgenic plant might be attributed to different mechanisms such as RNA co-suppression and not necessarily to the production of the protein sequence.

In general, the virus sequence will be transferred in an appropriate cell or tissue culture of the

plant species using an *Agrobacterium* mediated transformation system or a direct gene transfer method according to the constraints of the tissue culture or cell culture method which can be successfully applied in a given species. A whole plant will be regenerated and the expression of the transgene will be characterized.

Though sugar beet is known as a recalcitrant species in cell culture, limiting the extent of practical genetic engineering applications in that species, there are number of isolated reports of successful transformation and regeneration of whole plants (38). A few examples of engineering tolerance to the BNYVV by transforming and expressing the BNYVV coat-protein sequence in the sugar beet genome have also been published (39, WO91/13159) though they rarely report data on whole functional transgenic sugar beet plants (40). In particular, reports show limited data on the level of resistance observed in infected conditions with transgenic sugar beet plants transformed with a gene encoding a BNYVV coat-protein sequence (41, 42).

A complete technology package including a sugar beet transformation method and the use of the expression of the BNYVV coat-protein sequence as resistance source in the transgenic sugar beet plant obtained by said transformation method has been described in the Patent Application WO91/13159.

Based on the information published, it can not be concluded that the coat-protein mediated resistance mechanism provides any potential for conferring to the sugar beet plant a total immunity to the BNYVV-infection by inhibiting completely the virus multiplication and diffusion mechanisms. To identify a resistance mechanism which significantly blocks the spread of the virus at the early stage of the infection process would be a major step toward successfully developing such a transgenic

resistance. In addition, such resistance would diversify the mechanisms of resistance available.

Because the disease is shown to expand in many countries or areas, at a speed depending upon the combination of numerous local environmental and agricultural factors, there is a strong interest diversifying genetic resistance mechanisms which may, alone or in combination, confer a stable and long lasting resistance strategy in the current and future varieties of sugar beet plants which are grown for industrial use.

The genome of beet necrotic yellow vein benyvirus (BNYVV) consists of five plus-sense RNAs, two of which (RNAs 1 and 2) encode functions essential for infection of all plants while the other three (RNAs 3, 4 and 5) are implicated in vector-mediated infections of host plants (*Beta macrocarpa*, *Beta vulgaris*, *Spinacear oleracea*, *Chenopodium quinoa*, etc.) roots (1). Cell-to-cell movement of BNYVV is governed by a set of three successive, slightly overlapping viral genes on RNA 2 known as the triple gene block (TGB) (2), which encode the viral proteins P42, P13 and P15 (gene products are designated by their calculated M_r in kilodalton (3).

In the following description, the TGB genes and the corresponding proteins will be identified by the following terms: TGB1, TGB2, TGB3 or by their encoded viral protein number P42, P13 and P15. TGB counterparts are present in other plant viruses and the characteristics of their TGB have allowed the classification of said viruses in two groups: the viruses of group I which include hordéiviruses, benyviruses, pecluviruses and pomoviruses and the viruses of group II represented by potexviruses and carlaviruses (4, 5, 6, 44).

For the viruses of group II, capsid protein is also involved in the cell-to-cell movement of viruses.

The development of a resistance to viral infections into a plant by blocking the cell-to-cell movement has been described for the potato viruses X (PVX) (45) and for the white clover mosaic virus (WC1MV) (46) in *Nicotiana benthamiana*. These two viruses belong to the above-described group II. In both cases, various amino acids were replaced by Alanine in the hydrophilic part of the TGB sequence downstream of the N-terminal hydrophobic domain of said amino acid sequence. However, it was not possible with said mutants to obtain total resistance, especially when a virus challenger concentration is increasing into the plant.

Aims of the invention

The present invention aims to provide a new method for introducing various viral resistances into a cell and a plant and the viral resistant cell and plant obtained.

A main aim of the invention is to provide a new method for introducing BNYVV resistance into a cell and a plant and the BNYVV-resistant cell and plant, in particular a sugar beet cell and plant (*Beta vulgaris ssp.*), obtained.

Summary of the invention

The present invention provides the use of an alternative sequence of plant virus, especially the BNYVV, to obtain a high degree of tolerance to the viral infection, in particular to ensure a rapid and total blocking of virus multiplication and diffusion mechanisms in a plant, especially in the sugar beet plant (*Beta vulgaris*), including fodder beet, Swiss chard and table beet, which may also be subject to this viral infection. Expression of the resistance will be obtained in transgenic cell and plant, especially sugar beet cells and plants

produced by the transformation method subject to the Patent Application WO95/10178 or by other transformation methods based on *Agrobacterium tumefaciens* or direct gene transfer. Because of its high efficiency, the transformation method as described in WO95/10178 enables the production of large numbers of transformed plants, especially sugar beet plants, and will be preferred to develop transgenic plants which may be analysed and characterized for their level of viral resistance, especially BNYVV Resistance, including their field evaluation.

In the table 1 are represented viruses having a TGB2 sequence, the molecular weight of TGB2 of said viruses, their host and references.

Table 1

Virus	Size of TGB2 (kDa)	Host	Reference
GROUP I			
Beet necrotic yellow vein virus	13	beet	Bouzouba et al., *J. Gen. Virol.* 67, 1689-1700 (1986)
Barley stripe mosaic virus	14	barley	Gustafson et al., *Nucl. Acids Res.* 14, 3895-3909 (1986)
Potato mop top virus	13	potato	Scott et al., *J. Gen. Virol.* 75, 3561-3568 (1994)
Peanut clump virus	14	peanut	Herzog et al., *J. Gen. Virol.* 75, 3147-3155 (1994)
Beet soil-borne virus	13	sugar beet	Koenig et al., *Virology* 216, 202-207 (1996)
GROUP II			
Apple stem pitting virus	13	apple	Jelkman, *J. Gen. Virol.* 75, 1535-1542 (1994)
Blueberry scorch virus	12	blue-berry	Cavileer et al., *J. Gen. Virol.* 75, 711-720 (1994)
Potato virus M	12	potato	Zavriev et al., *J. Gen. Virol.* 72, 9-14 (1991)
White clover mosaic virus	13	clover	Forster et al., *Nucl. Acids Res.* 16, 291-303 (1988)
Cymbidium mosaic virus	14	orchid	Neo et al., *Plant Mol. Biol.* 18, 1027-1029 (1992)

The Inventors propose herewith a new method for providing resistance to plant viruses into a plant by blocking virus multiplication and diffusion mechanisms into said plant, especially into its root tissue. In order to demonstrate said resistance, the Inventors describe hereafter the effect of the overexpression of TGB2 sequence alone or in combination upon BNYVV multiplication and diffusion mechanism in plants of *C. quinoa* which are also

the hosts of the BNYVV virus and which could be more easily manipulated by the man skilled in the art.

It is known that BNYVV does not require synthesis of viral coat protein for production of local lesions on leaves of hosts such as *Chenopodium quinoa* (7), indicating that virion formation is not required for cell-to-cell movement.

However, the manner in which the TGB components assist in the movement process is not understood although computer-assisted sequence comparisons have detected characteristic conserved sequences which may provide clues to their function. Thus, the 5'-proximal TGB protein (TGB1) invariably contains a series of sequence motifs characteristic of an ATP/GTP-binding helicase while the second protein (TGB2) always has two potentially membrane-spanning hydrophobic domains separated by a hydrophilic sequence which contains a highly conserved peptide motif of unknown significance (6).

So far, no example has been reported of a virus of group I in which the three TGB members are arranged differently on the same RNA or are parcelled out to different genome RNAs, suggesting that their association in a particular order might be important in regulating their function.

The present invention concerns a method for inducing viral resistance to a virus of group I comprising the triple gene block (TGB2). Said viruses of group I comprise hordéiviruses, benyviruses, pecluviruses and pomoviruses, preferably viruses selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus; said method comprises the following steps:

- preparing a nucleotide construct comprising a nucleotide sequence corresponding to at least 70% of the wild-type

nucleotide sequence of TGB2 of said group I virus or its corresponding cDNA, being operably linked to one or more regulatory sequence(s) active in a plant,

- transforming a plant cell with the nucleotide construct, and possibly
- regenerating the transgenic plant from the transformed plant cell.

Advantageously, the nucleotide sequence corresponding to at least 70% of the wild-type nucleotide sequence of TGB2 or its corresponding cDNA comprise the substitution of at least one amino acid into another different amino acid in the TGB2 wild-type sequence SEQ ID NO. 1 (Fig. 1). Preferably, the substitution of at least one amino acid into another different amino acid is made in regions rich in hydrophilic amino acids usually present at the surface of the corresponding protein in its native configuration. Preferably, a modification is made in the hydrophilic region of the wild-type sequence downstream the N-terminal hydrophobic domain and just upstream the conserved central domain.

According to a preferred embodiment of the present invention, said amino acids are each substituted by the amino acid Alanine.

Preferably, the plant or plant cell is a plant or plant cell which may be infected by the above-described virus and is preferably selected from the group consisting of potato, barley, peanut and sugar beet.

The present invention concerns also the obtained plant cell and transgenic (or transformed) plant (made of said plant cells) resistant to said viruses and comprising said nucleotide construct.

The Inventors have also discovered unexpectedly that it is possible to induce BNYVV-resistance into a plant by a method which comprises the following steps:

- preparing a nucleotide construct comprising a nucleotide sequence corresponding to at least 70%, preferably at least 80%, more preferably at least 90%, of the wild-type nucleotide sequence comprised between the nucleotides 3287 and 3643 of the 5' strand of the genomic or subgenomic wild-type RNA 2 of the BNYVV or its corresponding cDNA, being operably linked to one or more regulatory sequence(s) active in a plant,
- transforming a plant cell with said construct, and possibly
- regenerating a transgenic plant from the transformed plant cell.

The nucleotide sequence comprised between the nucleotides 3287 and 3643 of the 5' strand of the genomic or subgenomic RNA 2 encoding the P13 protein is described in the Fig. 1 (SEQ ID NO. 1). A preferred mutated nucleotide sequence and its corresponding mutated amino acid sequence are described in the following specification as SEQ ID NO. 3 (Fig. 2).

Another aspect of the present invention concerns a plant cell and a transgenic plant (made of said plant cells) resistant to BNYVV and comprising a nucleotide construct having a nucleotide sequence corresponding to at least 70%, preferably at least 80%, more preferably at least 90%, of the nucleotide sequence comprised between the nucleotides 3287 and 3643 of the 5' strand of the genomic or subgenomic wild-type RNA 2 of BNYVV or its corresponding cDNA, being operably linked to one or more regulatory sequence(s) active in the plant.

Preferably, said plant cell or transgenic plant (made of said plant cells) resistant to BNYVV is obtained by the method according to the invention.

The variants of the wild-type nucleotide sequence (SEQ ID NO. 1) comprise insertion, substitution or deletion of nucleotides encoding the same or different

amino acid(s) (see Fig. 2). Therefore, the present invention concerns also said variants of the nucleotide sequence of SEQ ID NO. 1, for example SEQ ID NO. 3, which present at least 70%, preferably at least 80%, more preferably at least 90%, homology with said nucleotide sequence and which are preferably able to hybridise to said nucleotide sequence in stringent or non-stringent conditions as described by Sambrook et al., §§ 9.47-9.51 in *Molecular Cloning : A Laboratory Manual*, Cold Spring Harbor, Laboratory Press, Cold Spring Harbor, New York (1989).

A nucleotide sequence corresponding to at least 70%, preferably at least 80%, more preferably at least 90%, of the nucleotide sequence comprised between the nucleotides 3287 and 3643 of the 5' strand of the genomic or subgenomic wild-type RNA 2 of BNYVV or its corresponding cDNA, is preferably a sequence comprising a substitution of at least one amino acid into another different amino acid in the wild-type RNA2 sequence of the BNYVV or its corresponding cDNA. Preferably said substitution is made in regions in which hydrophilic amino acids are usually present at the surface of the protein in its native configuration (47) as described in Fig. 2 (A = substitution by Alanine). Preferably, said substitution of one or more amino acids is a mutation which allows the substitution of one or more amino acids into one or more Alanine amino acids.

According to a preferred embodiment of the present invention, said nucleotide sequence is SEQ ID NO. 3.

Preferably, said sequences are also able to induce BNYVV resistance into a plant.

The terms "induce a viral resistance into a plant" mean inducing a possible reduction or a significant delay into the appearance of infection symptoms, virus

multiplication or its diffusion mechanisms into the plant, especially in the root tissues.

In Fig. 3 are represented results showing the capacity of a plant coinoculated with virus containing a replicon construct with the nucleotide sequence according to the invention, especially the sequence SEQ ID NO. 3, to inhibit the movement by BNYVV in *C. Quinoa*. The infectious factor of BNYVV is shown by the appearance of local lesions of leaves of said plant after co-inoculation of wild-type virus S12. Fig. 3 presents the number of local lesions upon leaves of a plant by a BNYVV S12 isolate (comprising RNA1 and RNA2) when co-inoculated with various replicons incorporating either mutated sequences including SEQ ID NO. 3 identified in Fig. 2 or a wild-type nucleotide sequence (T).

Eight days after said inoculation, the local lesions are identified. The results of three experiments show that the decreasing of said effect is mostly observed with the co-inoculation of the mutated sequence SEQ ID NO. 3 (up to 100% inhibition). This effect is not due to a possible blocking effect upon RNA1 and RNA2 replication, but the replicons according to the invention allow a blocking of the biochemical mechanisms involved in cell-to-cell movements by the infectious virus.

The regulatory sequence(s) of the nucleotide sequence according to the invention are promoter sequence(s) and terminator sequence(s) active into a plant.

The nucleotide construct may also include a selectable marker gene, which could be used to identify the transformed cell or plant and express the nucleotide construct according to the invention.

Preferably, the cell is a stomatal cell and the plant is a sugar beet (*Beta vulgaris ssp.*) made of said cells.

According to the invention, the promoter sequence is a constitutive or foreigner promoter sequence. Examples are 35S Cauliflower Mosaic Virus promoter sequence, polyubiquitin *Arabidopsis thaliana* promoter (43), a promoter which is mainly active in root tissues such as the *par* promoter of the haemoglobin gene from *Perosponia andersonii* (Landsman et al., Mol. Gen. Genet. 214 : 68-73 (1988)) or a mixture thereof.

A last aspect of the present invention is related to a transgenic plant tissue such as fruit, stem, root, tuber, seed of the transgenic plant according to the invention or a reproducible structure (preferably selected from the group consisting of calluses, buds or embryos) obtained from the transgenic plant or the cell according to the invention.

The techniques of plant transformation, tissue culture and regeneration used in the method according to the invention are the ones well known by the person skilled in the art. Such techniques are preferably the ones described in the International Patent Applications WO95/10178 or WO91/13159 corresponding to the European Patent Application EP-B-0517833, which are incorporated herein by reference. These techniques are preferably used for the preparation of transgenic sugar beets according to the invention.

REFERENCES

1. Richards K.E. & Tamada T., *Annu. Rev. Phytopathol.* **30**, pp. 291-313 (1992)
2. Gilmer D. et al., *Virology* **189**, pp. 40-47 (1992)
3. Bouzoubaa S. et al., *J. Gen. Virol.* **68**, pp. 615-626 (1987)
4. Herzog E. et al., *J. Gen. Virol.* **18**, pp. 3147-3155 (1994)
5. Scott K. P. et al., *J. Gen. Virol.* **75**, pp. 3561-3568 (1994)
6. Koonin E.V. & Dolja V.V., *Crit. Rev. Biochem. and Mol. Biol.* **28**, pp. 375-430 (1993)
7. Schmitt C. et al., *Proc. Natl. Acad. Sci. USA.* **89**, pp. 5715-5719 (1992)
8. Morozov S.Y. et al., *J. Gen. Virol.* **72**, pp. 2039-2042 (1991)
22. Pelletier J. & Sonenberg N, *Nature* **334**, pp. 320-325 (1988)
23. Tamada T. & Baba T., *Annals of the Phytopathological Society of Japan* **39**, pp. 325-332 (1973)
24. Kuszala M.& Putz C., *Annals of Phytopathology* **9**, pp. 435-446 (1977)
25. Tamada T., *CMI/AAB Description of Plants Viruses* **44**, (1975)
26. Asher M.J.C., *Rhizomania* In *The sugar beet crop*, ed. D.A. Cooke and R.K. Scott, Chapman & Hall, London, pp. 312-338 (1993)
27. Richard-Molard M., *Rhizomanie* In *Institut français de la betterave industrielle. Compte-rendu des travaux effectués en* 1994, ITB, Paris pp. 225-229 (1995)
28. Henry C.M. et al, *Plant Pathology* **41**, pp. 483-489 (1992)
29. Grassi G. et al., *Phytopath. Medit.* **28**, pp. 131-139 (1989)

30. Merdinoglu D. et al., *Acad. Agric. Fr.* **79**, n° 6, pp. 85-98 (1993)

31. Scolten O.E. et al., *Archives of Virology* **136**, pp. 349-361 (1994)

32. Büttenr G. & Bürcky K., *Proceedings of the First Symposium of the International Working Group on Plant Viruses with Fungal* Vectors, Braunschweig Germany, August 21-24 (1990)

33. Whitney E.D., *Plant Disease* **73**, pp. 287-289 (1989)

34. Powell A.P. et al., *Science* **232**, pp. 738-743 (1986)

35. Fritchen J.H. & Beachy R.N., *Ann. Rev. Microbiol.* **47**, pp. 739-763 (1993)

36. Wilson T.M.A., *Proc. Natl. Acad. Sci. USA* **90**, pp. 3134-3141 (1993)

37. Gonsalves D. & Slightom J.L., *Seminars in Virology* **4**, pp. 397-405 (1993)

38. D'Halluin K. et al., *Biotechnology* **10**, pp. 309-314 (1992)

39. Kallerhof J. et al., *Plant Cell Reports 9*, pp. 224-228 (1990)

40. Ehlers U. et al., *Theoretical and Applied Genetic* **81**, pp. 777-782 (1991)

41. Kraus J. et al., *Field performance of transgenic sugar beet plants expresing BNYVV coat protein plants, Fourth International Congress of Plant Molecular Biology, Int. Soc. for Plant Molecular Biology*, Amsterdam (1994)

42. Maiss E. et al., *Proceedings of the Third International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms*, Monterey, pp. 129-139 (1994)

43. Norris et al., *Plant Molecular Biology* **21**, pp. 895-906 (1993)

44. Solovyev et al., *Virology* **219**, pp. 9-18 (1996)

45. Seppänen P. et al., *J. of General Virology* **78**, pp. 1241-1246 (1997)

46. Beck et al., *Proc. Natl. Acad. Sci. USA* **91**, pp. 10310-10314 (1994)

47. Cunningham et Wells, *Sciences* **244**, pp. 1081-1085 (1989)

CLAIMS

1. Method for inducing resistance to a group I virus comprising a TGB2 sequence in a plant cell or a plant, comprising the following steps:

- preparing a nucleotide construct comprising a nucleotide sequence having at least 70% homology with the nucleotide sequence of TGB2 of said virus or its complementary cDNA, being operably linked to one or more regulatory sequence(s) active in a plant,
- transforming a plant cell with the nucleotide construct, and possibly
- regenerating a transgenic plant from the transformed plant cell.

2. Method according to the claim 1, characterised in that the nucleotide sequence of the nucleotide construct has at least 80% homology with the nucleotide sequence of TGB2 of said virus or its complementary cDNA.

3. Method according to the claim 1 or 2, characterised in that the group I virus is selected from the group consisting of hordéiviruses, benyviruses, pecluviruses and pomoviruses, preferably selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus.

4. Method according to any of the preceding claims, characterised in that the plant cell is a stomatal cell.

5. Method according to any of the preceding claims, characterised in that the plant is selected from the group consisting of sugar beet, potato, barley or peanut.

6. Method according to claim 1 or 2, characterised in that the virus is BNYVV, the nucleotide sequence of TGB2 of said virus is comprised between the nucleotide 3287 and 3643 of the 5' strand of genomic or subgenomic RNA 2 of the BNYVV and the plant is a beet, preferably a sugar beet (*Beta vulgaris*).

7. Method according to any of the preceding claims, characterised in that the regulatory sequence comprises a promoter sequence or a terminator sequence active in a plant.

8. Method according to claim 7 characterised in that the promoter sequence is a constitutive or a foreigner promoter sequence.

9. Method according to the preceding claim 7, characterised in that the promoter sequence is selected from the group consisting of 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin Arabidopsis thaliana promoter.

10. Method according to any of the claim 7 to 9, characterised in that the promoter sequence is a promoter which is capable of being active mainly into the root tissues of plants, such as the par promoter of the haemoglobin gene from Perosponia andersonii.

11. Transgenic plant resistant to a group I virus comprising a nucleotide construct having at least 70% homology with the nucleotide sequence of TGB2 of said virus or its corresponding cDNA, being operably linked to one or more regulatory sequence(s) active in a plant.

12. Transgenic plant according to the claim 11, characterised in that the nucleotide construct has a

nucleotide sequence corresponding to at least 80% homology with the nucleotide sequence of TGB2 of said virus or its complementary cDNA.

13. Transgenic plant according to the claim 11 or 12, characterised in that the virus is selected from the group consisting of hordéiviruses, benyviruses, pecluviruses and pomoviruses, preferably selected from the group consisting of the beet necrotic yellow vein virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus and the beet soil-borne virus.

14. Transgenic plant according to the claims 11 to 13 being a plant selected from the group consisting of sugar beet, potato, barley or peanut.

15. Transgenic plant according to the claims 11 or 12, characterised in that the transgenic plant being a beet, preferably a sugar beet (*Beta vulgaris*) the virus is BNYVV and the nucleotide sequence of TGB2 of said virus is comprised between the nucleotides 3287 and 3643 of the 5' strand of genomic or subgenomic RNA 2 of BNYVV or its corresponding cDNA.

16. Transgenic plant according to any of the preceding claims 11 to 15, characterised in that the regulatory sequence comprises a promoter sequence and a terminator sequence active in a plant.

17. Transgenic plant according to any of the preceding claims 11 to 16, characterised in that the regulatory sequence(s) comprise a promoter sequence which is a constitutive or a foreign promoter sequence.

18. Transgenic plant according to the claim 17, characterised in that promoter sequence is selected from the group consisting of 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin *Arabidopsis thaliana* promoter.

19. Transgenic plant according to claim 17 or 18 characterised in that the promoter sequence is a promoter which is capable of being active mainly into root tissues, such as the *par* promoter of the haemoglobin gene from *Perosponia andersonii*.

20. Transgenic plant according to any one of the claims 11 to 19, characterised in that it further carries natural tolerance to Group I viruses.

21. Transgenic plant according to any one of the claims 11 to 20, characterised in that it further comprises a pesticide, herbicide or fungicide resistance, preferably a resistance selected from the group consisting of nematode resistance, glyphosate resistance, glufosomate resistance and/or acetochloride resistance.

22. Transgenic plant tissue selected from the group consisting of fruit, stem, root, tuber, seed of a plant according to any of the claims 11 to 21.

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[Continued on next page]

(54) Title: METHOD FOR INDUCING VIRAL RESISTANCE INTO A PLANT

```
3287 ATGTCTAGGGAAATAACCGCTCGACCCAATAAGAATGTGCCTATTGTTGTTGGTGTTTGT
     M  S  R  E  I  T  A  R  P  N  K  N  V  P  I  V  V  G  V  C  -

3347 GTTGTGGCTTTCTTTGTATTGCTGGCGTTCATGCAGCAAAAACATAAGACACATTCTGGG
     V  V  A  F  F  V  L  L  A  F  M  Q  Q  K  H  K  T  H  S  G  -

3407 GGTGATTACGGAGTCCCAACATTTTCTAACGGTGGTATATATAGAGACGGTACAAGATCA
     G  D  Y  G  V  P  T  F  S  N  G  G  I  Y  R  D  G  T  R  S  -

3467 GCTGATTTTAATAGTAACAATCATCGTGCTTACGGGTGCGGTGGGTCTGGGGGTAGCGTT
     A  D  F  N  S  N  N  H  R  A  Y  G  C  G  G  S  G  G  S  V  -

3527 AGTAGTCGAGTTGGGCAGCAACTTATTGTGTTAGCTATTGTTTCTGTGTTAATAGTGTCA
     S  S  R  V  G  Q  Q  L  I  V  L  A  I  V  S  V  L  I  V  S  -

3587 CTATTACAACGATTAAGGTCTCCACCAGAACACATTTGTAATGGTGCTTGTGGTTAA 3643
     L  L  Q  R  L  R  S  P  P  E  H  I  C  N  G  A  C  G  *
```

(57) Abstract: The present invention concerns a method for inducing resistance to a virus comprising a TGB2 sequence into a cell plant or a plant, comprising the following steps: preparing a nucleotide construct comprising a nucleotide sequence corresponding to at least 70 % of the nucleotide sequence of TGB2 of said virus or its complementary cDNA, being operably linked to one or more regulatory sequence(s) active in a plant, transforming a plant cell with the nucleotide construct, and possibly regenerating a transgenic plant from the transformed plant cell. The present invention is also related to the plant obtained.

```
3287 ATGTCTAGGGAAATAACCGCTCGACCCAATAAGAATGTGCCTATTGTTGTTGGTGTTTGT
     M  S  R  E  I  T  A  R  P  N  K  N  V  P  I  V  V  G  V  C  -

3347 GTTGTGGCTTTCTTTGTATTGCTGGCGTTCATGCAGCAAAAACATAAGACACATTCTGGG
     V  V  A  F  F  V  L  L  A  F  M  Q  Q  K  H  K  T  H  S  G  -

3407 GGTGATTACGGAGTCCCAACATTTTCTAACGGTGGTATATATAGAGACGGTACAAGATCA
     G  D  Y  G  V  P  T  F  S  N  G  G  I  Y  R  D  G  T  R  S  -

3467 GCTGATTTTAATAGTAACAATCATCGTGCTTACGGGTGCGGTGGGTCTGGGGGTAGCGTT
     A  D  F  N  S  N  N  H  R  A  Y  G  C  G  G  S  G  G  S  V  -

3527 AGTAGTCGAGTTGGGCAGCAACTTATTGTGTTAGCTATTGTTTCTGTGTTAATAGTGTCA
     S  S  R  V  G  Q  Q  L  I  V  L  A  I  V  S  V  L  I  V  S  -

3587 CTATTACAACGATTAAGGTCTCCACCAGAACACATTTGTAATGGTGCTTGTGGTTAA 3643
     L  L  Q  R  L  R  S  P  P  E  H  I  C  N  G  A  C  G  *
```

FIG. 1

```
3287 ATGTCTAGGGAAATAACCGCTCGACCCAATAAGAATGTGCCTATTGTTGTTGGTGTTTGT
     M  S  R  E  I  T  A  R  P  N  K  N  V  P  I  V  V  G  V  C  -

3347 GTTGTGGCTTTCTTTGTATTGCTGGCGTTCATGCAGCAAGCAGCTGCGACACATTCTGGG
     V  V  A  F  F  V  L  L  A  F  M  Q  Q  A  A  A  T  H  S  G  -

3407 GGTGATTACGGAGTCCCAACATTTTCTAACGGTGGTATATATAGAGACGGTACAAGATCA
     G  D  Y  G  V  P  T  F  S  N  G  G  I  Y  R  D  G  T  R  S  -

3467 GCTGATTTTAATAGTAACAATCATCGTGCTTACGGGTGCGGTGGGTCTGGGGGTAGCGTT
     A  D  F  N  S  N  N  H  R  A  Y  G  C  G  G  S  G  G  S  V  -

3527 AGTAGTCGAGTTGGGCAGCAACTTATTGTGTTAGCTATTGTTTCTGTGTTAATAGTGTCA
     S  S  R  V  G  Q  Q  L  I  V  L  A  I  V  S  V  L  I  V  S  -

3587 CTATTACAACGATTAAGGTCTCCACCAGAACACATTTGTAATGGTGCTTGTGGTTAA 3643
     L  L  Q  R  L  R  S  P  P  E  H  I  C  N  G  A  C  G  *
```

FIG. 2

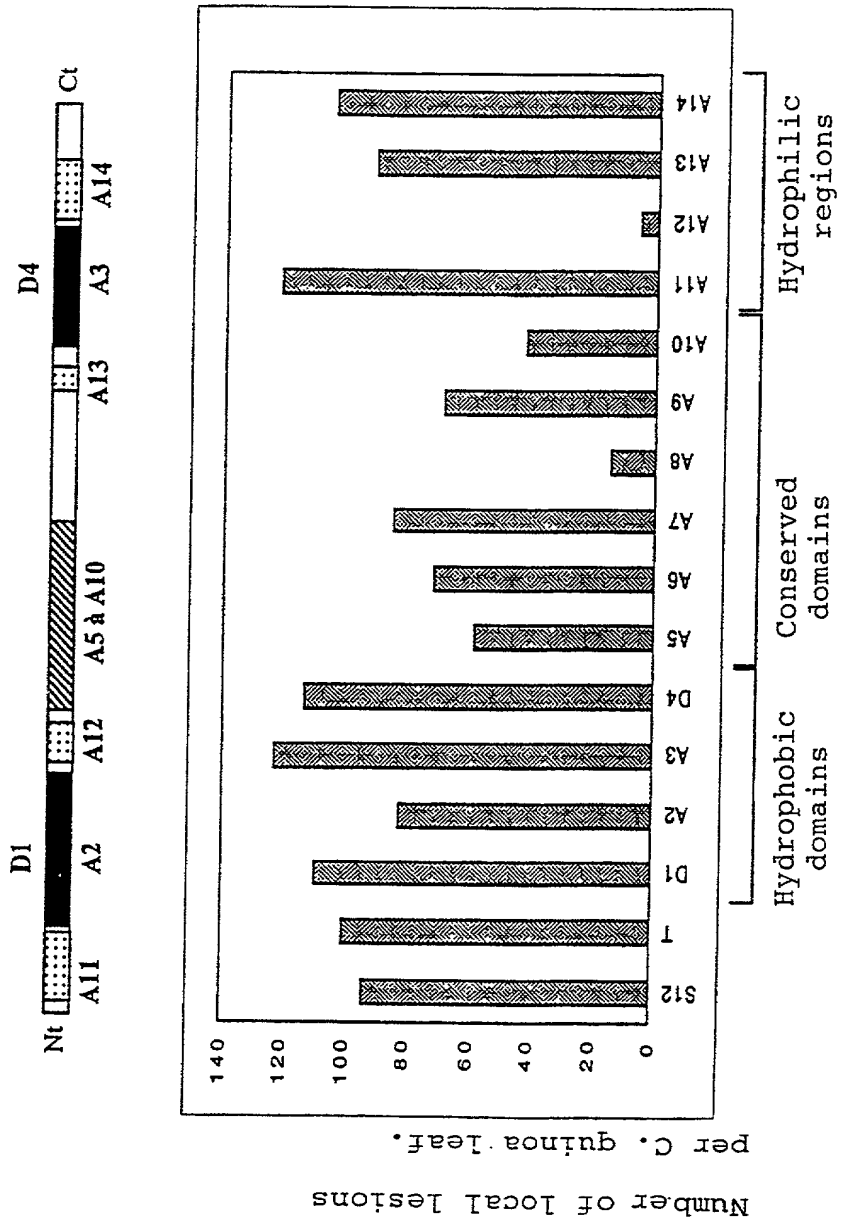
Nt
A11
D1
A2
A12
A5 à A10
D4
A3
A13
A14
Ct
Number of local lesions
per C. quinoa leaf.
140
120
100
80
60
40
20
0
S12
T
D1
A2
A3
D4
A5
A6
A7
A8
A9
A10
A11
A12
A13
A14
Hydrophobic domains
Conserved domains
Hydrophilic regions

Fig. 3

P.8ES.03/UPO

Declaration and Power of Attorney for Patent Application

Déclaration et Pouvoirs pour demandes de brevet

French Language Declaration

En tant que l'inventeur nommé ci-après, je déclare par le présent acte que :

As a below named inventor, I hereby declare that :

Mon domicile, mon adresse postale et ma nationalité figurant ci-dessous à côté de mon nom,

My residence, post office address and citizenship are as stated below next to my name,

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) du sujet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée :

I believe I am the original, first and sole (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled :

"METHOD FOR INDUCING VIRAL RESISTANCE INTO A PLANT"

et dont les caractéristiques sont fournies ci-joint à moins que la case suivante n'ait été cochée :

the specification of which is attached hereto unless the following box is checked :

O a été déposé le
sous le numéro de Demande des Etats-Unis ou sous le numéro de demande internationale PCT et modifiée le (le cas échéant).

O was filed on 7 March 2000
as United States Application Number or PCT International Application Number PCT/EP00/02176 and was amended on (if applicable).

Je déclare par le présent acte avoir passé en revue et pris connaissance du contenu des caractéristiques ci-dessus, revendications comprises, telles que modifiées par tout amendement dont il aura été fait référence ci-dessus.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Je reconnais de voir divulguer toute information pertinente à l'examen de cette demande, comme le définit le Titre 37, §1.56 du Code fédéral des réglementations.

I acknowledge the duty to disclose information which is material to patentibility as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119 du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur figurant ci-dessous et ai aussi pris connaissance de toute demande étrangère de brevet ou de tout certificat d'inventeur ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior foreign applications Demande(s) de brevet antérieure(s)		Priority claimed Droit de priorité revendiqué	
EP 99200773.2	EP		
(Number) (Numéro)	(Country) (Pays)	12 March 1999 (Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	☒ Yes / Oui ○ No / Non
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	○ Yes / Oui ○ No / Non
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	○ Yes / Oui ○ No / Non

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis figurant ci-dessous et, dans la mesure où le sujet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande américaine préalable, en vertu des dispositions de premier paragraphe du Titre 35, § 112 du Code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la demande de brevet comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la première demande et la date de dépôt de la demande nationale ou PCT internationale :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentibility as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application :

(Application Serial No.) (No. de série de la demande)	(Filing date) (Date de dépôt)	(Statut) (Breveté, en attente, annulé)	(Status) (Patented, pending, abandoned)
(Application Serial No.) (No. de série de la demande)	(Filing date) (Date de dépôt)	(Statut) (Breveté, en attente, annulé)	(Status) (Patented, pending, abandoned)

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'il(s) poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire avec le Bureau des brevets et marques s'y rapportant.
(mentionner le nom et le numéro d'enregistrement)

POWER OF ATTORNEY : As named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and trademark Office connected there with.

(list name and registration number)

Adresser toute correspondance à :

Send Correspondence to :

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone Calls to :
(name and telephone number)

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(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire)

(Supply similar information and signature for any subsequent joint inventor)

200

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Signature du troisième inventeur Date	Third inventor's signature Date August 16, 2001
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Signature du quatrième inventeur Date	Fourth inventor's signature Date August 16, 2001
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Nationalité	Citizenship French citizen
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SEQUENCE LISTING

<110> CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

<120> METHOD FOR INDUCING VIRAL RESISTANCE INTO A PLANT

<130> P.SES.03/EP

<140> 99200773.2
<141> 1999-03-12

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Val Gly Val Cys Val Val Ala Phe Phe Val Leu Leu Ala Phe Met Gln
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caa aaa cat aag aca cat tct ggg ggt gat tac gga gtc cca aca ttt   144
Gln Lys His Lys Thr His Ser Gly Gly Asp Tyr Gly Val Pro Thr Phe
         35                  40                  45

tct aac ggt ggt ata tat aga gac ggt aca aga tca gct gat ttt aat   192
Ser Asn Gly Gly Ile Tyr Arg Asp Gly Thr Arg Ser Ala Asp Phe Asn
     50                  55                  60

agt aac aat cat cgt gct tac ggg tgc ggt ggg tct ggg ggt agc gtt   240
Ser Asn Asn His Arg Ala Tyr Gly Cys Gly Gly Ser Gly Gly Ser Val
 65                  70                  75                  80

agt agt cga gtt ggg cag caa ctt att gtg tta gct att gtt tct gtg   288
Ser Ser Arg Val Gly Gln Gln Leu Ile Val Leu Ala Ile Val Ser Val
                 85                  90                  95

tta ata gtg tca cta tta caa cga tta agg tct cca cca gaa cac att   336
Leu Ile Val Ser Leu Leu Gln Arg Leu Arg Ser Pro Pro Glu His Ile
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<213> Beet necrotic yellow vein virus

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Gln Lys His Lys Thr His Ser Gly Gly Asp Tyr Gly Val Pro Thr Phe
         35                  40                  45

Ser Asn Gly Gly Ile Tyr Arg Asp Gly Thr Arg Ser Ala Asp Phe Asn
     50                  55                  60

Ser Asn Asn His Arg Ala Tyr Gly Cys Gly Gly Ser Gly Gly Ser Val
 65                  70                  75                  80

Ser Ser Arg Val Gly Gln Gln Leu Ile Val Leu Ala Ile Val Ser Val
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gtt ggt gtt tgt gtt gtg gct ttc ttt gta ttg ctg gcg ttc atg cag   96
Val Gly Val Cys Val Val Ala Phe Phe Val Leu Leu Ala Phe Met Gln
             20                  25                  30

caa gca gct gcg aca cat tct ggg ggt gat tac gga gtc cca aca ttt   144
Gln Ala Ala Ala Thr His Ser Gly Gly Asp Tyr Gly Val Pro Thr Phe
         35                  40                  45

tct aac ggt ggt ata tat aga gac ggt aca aga tca gct gat ttt aat   192
Ser Asn Gly Gly Ile Tyr Arg Asp Gly Thr Arg Ser Ala Asp Phe Asn
     50                  55                  60

agt aac aat cat cgt gct tac ggg tgc ggt ggg tct ggg ggt agc gtt   240
Ser Asn Asn His Arg Ala Tyr Gly Cys Gly Gly Ser Gly Gly Ser Val
 65                  70                  75                  80

agt agt cga gtt ggg cag caa ctt att gtg tta gct att gtt tct gtg   288
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                  85                  90                  95

tta ata gtg tca cta tta caa cga tta agg tct cca cca gaa cac att   336
Leu Ile Val Ser Leu Leu Gln Arg Leu Arg Ser Pro Pro Glu His Ile
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Gln Ala Ala Ala Thr His Ser Gly Gly Asp Tyr Gly Val Pro Thr Phe
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Ser Asn Gly Gly Ile Tyr Arg Asp Gly Thr Arg Ser Ala Asp Phe Asn
     50                  55                  60

Ser Asn Asn His Arg Ala Tyr Gly Cys Gly Gly Ser Gly Gly Ser Val
 65                  70                  75                  80

Ser Ser Arg Val Gly Gln Gln Leu Ile Val Leu Ala Ile Val Ser Val
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Leu Ile Val Ser Leu Leu Gln Arg Leu Arg Ser Pro Pro Glu His Ile
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Cys Asn Gly Ala Cys Gly
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